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Yamamoto , et al. **January 18, 2000**

Plant VDE genes and methods related thereto

Abstract

DNA sequences encoding plant vde enzymes are provided herein. The sequences may be joined to heterologous DNA sequences for use as probes and in DNA constructs to modify the genotype of a host organism. DNA constructs and methods are provided to modify a host cell phenotype by altering the amount of photoprotection enzyme present in the host cell. In plastid containing host cells, zeaxanthin levels and sensitivity to light can be modified through alterations in the level of vde enzymes.

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Parent Case Text

This application claims benefit of provisional application No. 60,023,502, filed Aug. 6, 1996, which is a provisional of 60,006,315, filed Nov. 7, 1995.

Claims

1. An isolated DNA sequence encoding plant violaxanthin de-epoxidase.

2. The DNA sequence of claim 1 wherein said violaxanthin de-epoxidase DNA sequence is joined to a heterologous nucleic acid sequence.

3. A recombinant DNA construct capable of directing the transciption of RNA in a plant cell, wherein said construct comprises in the order of transcription, a plant transcription initiation region, the violaxanthin de-epoxidase encoding sequence of claim 1, and a transcriptional termination region.

4. The DNA sequence of claim 1, wherein said sequence is selected from the group consisting of the nucleic acid sequences in SEQ ID NO 1, SEQ ID NO 2 and SEQ ID NO 3.

5. The DNA sequence of claims 1, wherein said sequence encodes at least about the twenty N-terminus amino acids of a protein selected from the group consisting of the plant violaxanthin de-epoxidase proteins in SEQ ID NO 1, SEQ ID NO 2 and SEQ ID NO 3.

6. The DNA sequence of claim 5, wherein said sequence encodes a plant violaxanthin de-epoxidase protein selected from the group consisting of the proteins in SEQ ID NO. 1, SEQ ID NO 2, and SEQ ID NO 3.

7. The DNA sequence of claim 1, wherein said sequence encodes a protein comprising the amino acids VDALKTCACLLK.

8. A method of modifying the violaxanthin de-epoxidase levels in a plant, said method comprising growing a plant transformed by a construct according to claim 3.

9. The method of claim 8 wherein said encoding sequence is in sense orientation.

10. The method of claim 9 wherein said construct further comprises a plastid translocation sequence.

11. A method of modifying the sensitivity of a transgenic plant to light comprising growing a plant transformed by a construct according to claim 3.

12. The method of claim 10 wherein violaxanthin de-epoxidase activity is increased resulting in increased zeaxanthin and antheraxanthin production.

13. The method of claim 12 wherein said increased zeaxanthin and antheraxanthin levels results in said plant being tolerant of increased light levels, as opposed to a non-transformed control plant of the same type.

14. A transgenic plant with modified sensitivity to light as a consequence of the activity of an introduced construct according to claim 3.

15. A plant, plant cell or other plant part, each comprising a construct according to claim 3.

16. A plant, plant cell or other plant part, each produced by the method of claim 8.

17. A plant, plant cell or other plant part, each produced by the method of claim 10 wherein flowering of said plant is delayed as compared to flowering in a control plant not produced by said method.

18. A plant, plant cell or other plant part, each produced by the method of claim 10 wherein flowers of said plant are larger as compared to flowers of a control plant not produced by said method.

Description

FIELD OF THE INVENTION

This invention relates to genes encoding plant violaxanthin de-epoxidase (vde) and methods of use related to the protein and the nucleic acid sequences. The invention is exemplified by methods of

causing increased expression or decreased expression of plant vde genes in plants. Included are plants produced by the method.

INTRODUCTION

Background

Plant carotenoids are found in the membranes of chloroplasts and chromoplasts. They are instrumental in the photoprotective mechanisms of plants. Also, plant carotenoids have significant dietary implications. Thus, from an agronomic as well as a nutritional standpoint, study of the plant carotenoids and the enzymes involved in the biosynthesis of carotenoids is of interest.

Of particular interest are the late stages of the carotenoid biosynthetic pathway in plants, the xanthophyll cycle and its importance in photoregulation of photosynthesis. Photosynthesis is the process that enable plants to use light energy for growth and development. Thus, the availability of light of appropriate quality and quantity (photosynthetically active radiation or "PAR") is critical for plant growth and development. Ironically, light can also damage plants because plants have limited capacity to use light. When light intensity exceeds this capacity, irreversible damage can occur.

Plants have developed various mechanisms to cope with excess light such as varying leaf orientation or developing reflective surfaces. Such mechanisms appear to be specialized phenotypic strategies that are limited to certain types of plants. One mechanism that is apparently used by all plants examined so far is the dissipation of excess energy as heat in the antenna (light absorbing structures) of the photosynthetic apparatus. Most of the excess energy is discarded as heat by a complex feed-back regulatory system that involves the transthylakoid .DELTA.pH and formation of antheraxanthin and zeaxanthin catalyzed by violaxanthin de-epoxidase (vde) in the xanthophyll cycle. This system, termed energy dependent non-radiative energy dissipation or non-photochemical fluorescence quenching, reduces the quantum efficiency of photosystem II (PSII), helping to prevent PSII over reduction and photoinhibitory damage. In effect, this system provides a means to dump excess energy before it can damage the photosynthetic apparatus. The system has a wide dynamic range, both qualitatively and quantitatively, which enables it to function effectively over a wide-range of environmental conditions.

The ability to manipulate aspects of the xanthophyll cycle through genetic engineering techniques would permit the rapid introduction of improved plant varieties. However, it has been difficult to obtain purified fractions of the enzymes involved in the pathway and, prior to this invention, the corresponding genes have not been cloned.

SUMMARY OF THE INVENTION

DNA sequences encoding plant vde enzymes are provided herein. The sequences may be joined to heterologous DNA sequences for use as probes and in DNA constructs to modify the genotype of a host organism. DNA constructs and methods are provided to modify a host cell phenotype by altering the amount of photoprotection enzyme present in the host cell. In plastid containing host cells, zeaxanthin levels and sensitivity to light can be modified through alterations in the level of vde enzymes.

For example, over expression of vde is expected to increase the tolerance of plants to high light, drought and temperature stress (stress conditions exacerbate the condition of excess light). Also, plants that are not currently tolerant to high light or low temperatures are expected to become more tolerant to these stresses. Plants that are better adapted to light stress are expected to be more